Gluconobacter japonicus sp. nov., an acetic acid bacterium in the *Alphaproteobacteria*

Taweesak Malimas,¹ Pattaraporn Yukphan,¹ Mai Takahashi,²
Yuki Muramatsu,² Mika Kaneyasu,² Wanchern Potacharoen,¹
Somboon Tanasupawat,³ Yasuyoshi Nakagawa,² Morakot Tanticharoen¹
and Yuzo Yamada^{1†}

Correspondence

Yuzo Yamada

yamada333@kch.biglobe.ne.jp

¹BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani 12120, Thailand

²Biological Resource Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation (NITE), Kisarazu 292-0818, Japan

³Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

Five strains, NBRC 3271^T, NBRC 3272, NBRC 3263, NBRC 3260 and NBRC 3269 were examined genetically, phylogenetically, phenotypically and chemotaxonomically. The DNA G+C contents of the five strains were 55.1–56.4 mol%. The five strains had low levels of DNA–DNA hybridization of 13–51 % to the type strains of *Gluconobacter frateurii*, *Gluconobacter thailandicus*, *Gluconobacter oxydans*, *Gluconobacter cerinus*, *Gluconobacter albidus* and *Gluconobacter kondonii* and formed a cluster that was separate from the type strains of the six *Gluconobacter* species given above in phylogenetic trees based on 16S rRNA gene and 16S–23S rRNA gene internal transcribed spacer sequences. The five strains weakly produced dihydroxyacetone from glycerol, but not 2,5-diketo-D-gluconate or a water-soluble brown pigment from D-glucose and contained ubiquinone-10. The five strains were assigned as representing a novel species of the genus *Gluconobacter*, for which the name *Gluconobacter japonicus* sp. nov. is proposed. The type strain is NBRC 3271^T (=BCC 14458^T=strain 7^T, K. Kondo). Cells of the type strain are motile by means of polar flagella and the DNA G+C content is 56.4 mol%.

Members of the genus *Gluconobacter* are characterized physiologically as not being able to oxidize acetate and lactate and chemotaxonomically by having ubiquinone-10 (Q-10) as a major quinone (Asai, 1935; Asai *et al.*, 1964; Yamada *et al.*, 1969; Gosselé *et al.*, 1983). The combination of the phenotypic and the chemotaxonomic characteristics has been used to classify a large number of isolated acetic acid bacteria as belonging to the genus *Gluconobacter*

(Yamada *et al.*, 1999; Tanaka *et al.*, 1999; Huong *et al.*, 2007).

At the time of writing, the genus *Gluconobacter* contained seven species with validly published names: *G. oxydans* (the type species), *G. cerinus*, *G. frateurii*, *G. asaii*, *G. albidus*, *G. thailandicus* and *G. kondonii* (De Ley, 1961; Skerman *et al.*, 1980; Gosselé *et al.*, 1983; Yamada & Akita, 1984a, b; Mason & Claus, 1989; Yukphan *et al.*, 2004c, 2005; Tanasupawat *et al.*, 2004, 2005; Malimas *et al.*, 2007, 2008). *G. asaii* is considered to be a later heterotypic synonym of *G. cerinus* (Katsura *et al.*, 2002; Yamada *et al.*, 1999; Tanaka *et al.*, 1999).

The heterogeneity of strains assigned to *G. frateurii* was recognized by Malimas *et al.* (2006), based on 16S–23S rRNA gene internal transcribed spacer (ITS) restriction and sequence analyses. In a restriction analysis of the 16S–23S rRNA gene ITS with six restriction endonucleases, the strains were largely divided into two groups, Group III and Group IV. However, Group III was further divided into Group III-1, Group III-2, Group III-3, Group III-4 and Group III-5. In a phylogenetic tree based on 16S–23S rRNA

†JICA Senior Overseas Volunteer, Japan International Cooperation Agency (JICA), Shibuya-ku, Tokyo 151-8558, Japan; Professor Emeritus, Shizuoka University, Shizuoka 422-8529, Japan.

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains NBRC 3271^T, NBRC 3260, NBRC 3263, NBRC 3272 and NBRC 3269 are AB253435, AB178400, AB253434, AB253436 and AB178408, respectively.

A table showing DNA–DNA hybridization values for strains NBRC 3271^T, NBRC 3260, NBRC 3263, NBRC 3272 and NBRC 3269 and the type strains of other species of *Gluconobacter* and *Acetobacter aceti* is available as supplementary material with the online version of this paper.

gene ITS sequences, the strains constituted three large clusters: (i) a large cluster that included strains of Group III-2, including the type strain of *G. frateurii*, Group IV and Subgroup III-1a; (ii) a second large cluster that included strains of Group III-3, including the type strain of *G. thailandicus*, and Subgroup III-1b; (iii) a third large cluster that included five strains of Group III-5, Subgroup III-4a and Subgroup III-4b. Phylogenetically, the five strains of the third large cluster were suggested to constitute a new taxon separate from either *G. frateurii* or *G. thailandicus*.

A similar cluster, designated Cluster 1–2, was recognized by Takahashi *et al.* (2006) in a phylogenetic tree based on 16S–23S rRNA gene ITS sequences. The five strains examined in this paper were included in this cluster and it was suggested that the strains should be classified as representing *G. frateurii* or a novel species.

In this paper we propose the name *Gluconobacter japonicus* sp. nov. to accommodate the five strains grouped into Group III-5, Subgroup III-4a and Subgroup III-4b.

The five strains, NBRC 3271^T (received as *G. frateurii* from NBRC), NBRC 3272 (received as *G. frateurii*) and NBRC 3263 (received as *G. cerinus*) of Group III-5, NBRC 3260 (received as *G. frateurii*) of Subgroup III-4a and NBRC 3269 (received as *G. cerinus*) of Subgroup III-4b were examined genetically, phylogenetically, phenotypically and chemotaxonomically. *G. frateurii* NBRC 3264^T, *G. thailandicus* BCC 14116^T, *G. oxydans* NBRC 14819^T, *G. cerinus* NBRC 3267^T, *G. albidus* NBRC 3250^T, *G. kondonii* NBRC 3266^T and *Acetobacter aceti* NBRC 14818^T were used as reference strains.

Extraction and isolation of bacterial chromosome DNA were performed by using a modification of the method of Marmur (1961) (Saito & Miura, 1963; Ezaki *et al.*, 1983). The DNA base composition was determined by using the method of Tamaoka & Komagata (1984). The DNA G+C contents of strains NBRC 3271^T, NBRC 3272, NBRC 3263, NBRC 3260 and NBRC 3269, respectively, were 56.4, 56.4, 56.4, 55.1 and 55.7 mol%, with a range of 1.3 mol%. The data obtained indicated that the five strains are included in a low DNA G+C content group, phenon A or a sublineage of *G. crinus*/*G. frateurii*, along with the type strains of *G. cerinus*, *G. frateurii* and *G. thailandicus* (Yamada & Akita, 1984a; Gossele *et al.*, 1983; Yamada *et al.*, 1984, 2000; Mason & Claus, 1989; Tanasupawat *et al.*, 2004).

DNA–DNA hybridization was carried out by using the photobiotin-labelling method with microplate wells, as described by Ezaki *et al.* (1989) (Yukphan *et al.*, 2004c, d; Malimas *et al.*, 2007). Levels of DNA–DNA hybridization were determined colorimetrically (Verlander, 1992). The colour intensity was measured at A₄₅₀ using a model VersaMax microplate reader (Molecular Devices). When single-stranded and labelled DNA from strain NBRC 3271^T was hybridized with DNA from the test strains in 2 × SSC containing 50 % formamide at 49.0 °C for 15 h, levels of DNA–DNA hybridization of 100, 100, 100, 100, 77, 48, 49,

29, 23, 17, 16 and 6 %, respectively, were obtained with DNA of the five strains (NBRC 3271^T, NBRC 3272, NBRC 3263, NBRC 3260 and NBRC 3269) and *G. frateurii* NBRC 3264^T, *G. thailandicus* BCC 14116^T, *G. cerinus* NBRC 3267^T, *G. oxydans* NBRC 14819^T, *G. albidus* NBRC 3250^T, *G. kondonii* NBRC 3266^T and *A. aceti* NBRC 14818^T. In addition, labelled DNA from *G. frateurii* NBRC 3264^T and *G. thailandicus* BCC 14116^T showed DNA–DNA hybridization values of, respectively, 53, 51, 50, 57, 49, 100, 54, 27, 15, 15, 11 and 4 % and 49, 43, 39, 44, 40, 48, 100, 24, 16, 15, 12 and 5 %. Among the five strains studied, the calculated DNA–DNA hybridization levels were 70–100 %. Labelled DNA from strains NBRC 3272, NBRC 3263, NBRC 3260 and NBRC 3269 had levels of DNA–DNA hybridization of 34–48, 33–51, 19–29, 19–23, 12–19, 13–19 and 4–6 %, respectively, to *G. frateurii* NBRC 3264^T, *G. thailandicus* BCC 14116^T, *G. cerinus* NBRC 3267^T, *G. oxydans* NBRC 14819^T, *G. albidus* NBRC 3250^T, *G. kondonii* NBRC 3266^T and *A. aceti* NBRC 14818^T. The detailed DNA–DNA hybridization results are available in Supplementary Table S1 (in IJSEM Online). The data obtained indicate that, genetically, the five strains can be separated at the species level from the type strains of the six *Gluconobacter* reference species used.

Micales *et al.* (1985) found high levels of DNA–DNA hybridization of 77 and 87 %, respectively, to strains IFO 3271^T (=NBRC 3271^T) and IFO 3272 (=NBRC 3272), when hybridized with labelled DNA from strain IFO 3264^T (=NBRC 3264^T). However, Mason & Claus (1989) did not designate the two strains as representing *G. frateurii*. On the other hand, Tanaka *et al.* (1999) found low levels of DNA–DNA hybridization of 45, 56, 50 and 53 %, respectively, for strains IFO 3271^T (=NBRC 3271^T), IFO 3263 (=NBRC 3263), IFO 3260 (=NBRC 3260) and IFO 3269 (=NBRC 3269) with labelled DNA from the type strain of *G. frateurii*. Considering the phylogenetic relationships represented previously by Malimas *et al.* (2006) and Takahashi *et al.* (2006) and as shown below that the two strains, NBRC 3271^T (=IFO 3271^T) and NBRC 3272 (IFO 3272), constituted a third large cluster, the low levels of DNA–DNA hybridization of 45–56 % calculated by Tanaka *et al.* (1999) were reasonable from the phylogenetic point of view.

Phylogenetic analyses based on 16S rRNA gene sequences and 16S–23S rRNA gene ITS sequences were performed for the five strains, as described previously (Yukphan *et al.*, 2004b, d; Malimas *et al.*, 2006, 2007). Multiple sequence alignments were made using the program CLUSTAL_X (version 1.81) (Thompson *et al.*, 1997). Sequence gaps and ambiguous bases were excluded. Distance matrices were calculated by using the two-parameter method of Kimura (1980). The neighbour-joining method was used for constructing phylogenetic trees (Saitou & Nei, 1987). In addition, the maximum-parsimony and the maximum-likelihood methods were used (Felsenstein, 1981, 1983). The robustness of individual branches was estimated by bootstrapping based on 1000 replications (Felsenstein,

Fig. 1. Phylogenetic relationships of strains NBRC 3271^T, NBRC 3272, NBRC 3263, NBRC 3260 and NBRC 3269 (*G. japonicus* sp. nov.). The phylogenetic tree was based on 16S rRNA gene sequences and was constructed by using the neighbour-joining method. The type strain of *Acetobacter acetii* was used as an outgroup. Numbers at nodes indicate bootstrap percentages derived from 1000 replications. Bar, 0.005 K_{nuc} .

1985) with the program MEGA (version 4.0; Tamura *et al.*, 2007). In particular, the program PHYLIP (version 3.6; Felsenstein, 1995) was used to construct a phylogenetic tree using the maximum-likelihood method.

In a phylogenetic tree based on 16S rRNA gene sequences of 1428 nt constructed using the neighbour-joining method, the five strains formed a cluster that was separate from the type strains of *G. frateurii* and *G. thailandicus* (Fig. 1) (Takahashi *et al.*, 2006; Malimas *et al.*, 2006). In phylogenetic trees constructed using the maximum-parsimony and maximum-likelihood methods, similar clustering was found (data not shown). In a phylogenetic tree based on 16S–23S rRNA gene ITS sequences of 691 nt, constructed using the neighbour-joining method, the five strains formed a cluster that was similar to that obtained based upon 16S rRNA gene sequences (data not shown) (Malimas *et al.*, 2006). The data above indicate that the five strains can be separated phylogenetically from the type strains of the six *Gluconobacter* species studied.

Pairwise 16S rRNA gene sequence similarities of strain NBRC 3271^T (for 1430 nt) were calculated to be 100, 100, 99.9, 99.9, 99.9, 99.7, 99.2, 98.0, 97.9 and 97.9%, respectively, to strains NBRC 3272, NBRC 3263, NBRC 3260 and NBRC 3269 and the type strains of *G. frateurii*, *G. thailandicus*, *G. cerinus*, *G. oxydans*, *G. albidus* and *G. kondonii*. The pairwise sequence similarities between the five strains were 99.9–100%. Using the 16S–23S rRNA gene ITS sequences, the calculated pairwise sequence similarities of strain NBRC 3271^T (747 nt) were, respectively, 99.9, 99.9, 99.3, 99.7, 97.9, 97.4, 95.8, 83.4, 82.7 and 82.6%. The pairwise sequence similarities between the five strains were 99.3–100%. The calculated pairwise 16S rRNA gene sequence similarities of 99.9 and 99.7%, respectively, between strain NBRC 3271^T and the type strain of *G. frateurii* and between strain NBRC 3271^T and the type strain of *G. thailandicus* were high. However, based on the DNA–DNA hybridization data detailed above, strain NBRC 3271^T was separate genetically at the species level.

As the five strains studied could be distinguished from the type strain of either *G. frateurii* or *G. thailandicus* in the

low DNA G + C content group by the 16S–23S rRNA gene ITS restriction analysis using *TaqI* (Malimas *et al.*, 2006), the 16S–23S rRNA gene ITS PCR products of the five strains were prepared and analysed by digestion with *MboII*, *BspI286I* and *BstNI* in addition to *TaqI* (Trček & Teuber, 2002; Yukphan *et al.*, 2004a, b; Malimas *et al.*, 2006). As shown in Fig. 2(a, b), the five strains could be distinguished from the type strains of *G. oxydans*, *G. cerinus*, *G. albidus* and *G. kondonii* using *MboII* and *BspI286I* digestions. In addition, the five strains could be distinguished from the type strains of *G. frateurii* and *G. thailandicus* by *TaqI* and *BstNI* digestions (Fig. 2c, d). The data given above indicate that the five strains can be discriminated from the type strains of the six *Gluconobacter* species used by using 16S–23S rRNA gene ITS restriction analysis with *MboII*, *BspI286I*, *TaqI* and *BstNI*.

The morphological, physiological and biochemical characteristics of the five strains were examined as described previously (Yamada *et al.*, 1976, 1999; Katsura *et al.*, 2002) and by Asai *et al.* (1964), Gossele *et al.* (1983) and Mason & Claus (1989). The ubiquinone system was determined by using the method of Yamada *et al.* (1969). The phenotypic and chemotaxonomic characteristics determined are given in the species description.

The five strains could be discriminated from the type strains of *G. oxydans*, *G. albidus* and *G. kondonii*, which are grouped in the high DNA G + C content group, by growth in the presence of L-arabitol (weakly positive in strains NBRC 3271^T, NBRC 3272 and NBRC 3263) and meso-ribitol, as well as by the ability to grow without nicotinic acid (Table 1). The five strains weakly produced dihydroxyacetone from glycerol, different from the type strains of *G. frateurii*, *G. thailandicus*, *G. cerinus*, *G. oxydans*, *G. albidus* and *G. kondonii*. The five strains produced acid from meso-erythritol [different from the type strains of *G. frateurii* (weakly positive) and *G. albidus* (weakly positive)] and from raffinose [different from the type strains of *G. thailandicus* (weakly positive), *G. cerinus* (weakly positive) and *G. oxydans* (weakly positive)], but not from maltose (weakly positive in strains NBRC 3260 and NBRC 3269), different

Fig. 2. Digestion of 16S–23S rRNA gene ITS of strains NBRC 3271^T, NBRC 3272, NBRC 3263, NBRC 3260 and NBRC 3269 (*G. japonicus* sp. nov.) with restriction endonucleases. The restriction patterns were obtained by digestion of 16S–23S rRNA gene ITS PCR products with *Mbo*II (a), *Bsp*1286I (b), *Taq*I (c) and *Bst*NI (d). For estimation of the resulting restriction fragments, 50 bp DNA markers were used in agarose gel electrophoresis. Lanes: 1, *G. frateurii* NBRC 3264^T; 2, *G. thailandicus* BCC 14116^T; 3, strain NBRC 3271^T; 4, strain NBRC 3272; 5, strain NBRC 3263; 6, strain NBRC 3260; 7, strain NBRC 3269; 8, *G. cerinus* NBRC 3267^T; 9, *G. oxydans* NBRC 14819^T; 10, *G. albidus* NBRC 3250^T; 11, *G. kondonii* NBRC 3266^T; M, 50 bp DNA marker.

from the type strains of *G. frateurii* (weakly positive) and *G. oxydans*. The five strains grew on *meso*-erythritol, different from the type strains of *G. frateurii*, *G. thailandicus* (weakly positive), *G. oxydans* (weakly positive) and *G. albidus*. In growth on pentitol, the five strains grew on D-arabitol (different from the type strains of *G. oxydans* and *G. albidus*) and on *meso*-ribitol [different from the type strains of *G. thailandicus* (weakly positive), *G. cerinus* (weakly positive), *G. oxydans*, *G. albidus* and *G. kondonii*].

The five strains could be discriminated from the type strains of *G. oxydans*, *G. albidus*, *G. kondonii* and *G. cerinus* by 16S–23S rRNA gene ITS restriction analysis with *Mbo*II and *Bsp*1286I (Table 1) (Yukphan *et al.* 2004a, b). In addition, the five strains represented a different kind of restriction pattern especially from those of the type strains of *G. frateurii* and *G. thailandicus* by digestion with *Taq*I (Malimas *et al.*, 2006).

As described above, the five strains studied could be distinguished genetically, phylogenetically, morphologically, physiologically, biochemically and chemotaxonically from the type strains of *G. oxydans*, *G. albidus*, *G. kondonii*, *G. cerinus*, *G. frateurii* and *G. thailandicus* (Table 1). A novel species is therefore proposed to accommodate the five strains grouped into the low DNA G + C content group, phenon A or the sublineage of *G. cerinus*/*G. frateurii*, with the name *Gluconobacter japonicus* sp. nov.

Description of *Gluconobacter japonicus* sp. nov.

Gluconobacter japonicus (ja.po.ni'cus. N.L. masc. adj. *japonicus* of Japan, where the type strain was isolated).

Cells are Gram-negative rods, measuring 0.8–1.0 × 1.0–2.0 µm. Variable results are obtained for motility and the presence of polar flagella. Grows at pH 3.0 and 35 °C. Maximum growth occurs at pH 5.5 and 25 °C. Does not oxidize acetate or lactate. Grows on mannitol agar but not on glutamate agar. Produces dihydroxyacetone (weakly positive) from glycerol and 2-keto-D-gluconate and 5-keto-D-gluconate from D-glucose, but not 2,5-diketo-D-gluconate or a water-soluble brown pigment from D-glucose. Acid is produced from D-glucose, D-mannose, D-galactose, D-xylose, D-arabinose, L-arabinose, L-rhamnose (weakly positive), D-fructose, L-sorbose, D-mannitol, D-sorbitol, *meso*-erythritol, glycerol, melibiose, sucrose, raffinose and ethanol. Acid is not produced from dulcitol or lactose. Variable results are obtained for acid production from maltose. Grows on D-glucose, D-fructose, D-mannitol, D-sorbitol, *meso*-erythritol and glycerol. Variable results are obtained for growth on D-arabinose, L-arabinose, L-rhamnose, L-sorbose, sucrose and raffinose. Does not grow on D-mannose, D-galactose, D-xylose, dulcitol, maltose, melibiose, lactose or ethanol. Grows on pentitol with D-arabitol and *meso*-ribitol. Variable results are obtained for growth on pentitol with L-arabitol. Does not require

Table 1. Characteristics that differentiate strains NBRC 3271^T, NBRC 3272, NBRC 3263, NBRC 3260 and NBRC 3269 (*Gluconobacter japonicus* sp. nov.) from the type strains of the six *Gluconobacter* species studied

Strains: 1, NBRC 3271^T; 2, NBRC 3272; 3, NBRC 3263; 4, NBRC 3260; 5, NBRC 3269; 6, *G. frateurii* NBRC 3264^T; 7, *G. thailandicus* BCC 14116^T; 8, *G. cerinus* NBRC 3267^T; 9, *G. oxydans* NBRC 14819^T; 10, *G. albidus* NBRC 3250^T; 11, *G. kondonii* NBRC 3266^T. +, Positive; –, negative; w, weakly positive; ND, not determined; *Gf*, *G. frateurii*; *Gc*, *G. cerinus*; *Go*, *G. oxydans*; *Ga*, *G. albidus*; *Gk*, *G. kondonii*.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Production of dihydroxyacetone	w	w	w	w	w	+	+	+	+	+	+
Acid production from:											
<i>meso</i> -Erythritol	+	+	+	+	+	w	+	+	+	w	+
Maltose	–	–	–	w	w	w	–	–	+	–	–
Raffinose	+	+	+	+	+	+	w	w	w	+	+
Growth on:											
<i>meso</i> -Erythritol	+	+	+	+	+	–	w	+	w	–	+
D-Arabitol	+	+	+	+	+	+	+	+	–	–	+
L-Arabitol	w	w	w	+	+	+	w	–	–	–	–
<i>meso</i> -Ribitol	+	+	+	+	+	+	w	w	–	–	–
Growth without nicotinic acid	+	+	+	+	+	+	+	+	–	–	–
Restriction pattern with*											
<i>Mbo</i> II	<i>Gf</i>	<i>Gf</i>	<i>Gf</i>	<i>Gf</i>	<i>Gf</i>	<i>Gf</i>	<i>Gf</i>	<i>Gc</i>	<i>Go</i>	<i>Ga</i>	<i>Gk</i>
<i>Bsp</i> 1286I	<i>Gf</i>	<i>Gf</i>	<i>Gf</i>	<i>Gf</i>	<i>Gf</i>	<i>Gf</i>	<i>Gf</i>	<i>Gc</i>	<i>Go</i>	<i>Go</i>	<i>Gk</i>
<i>Taq</i> I	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>c</i>	<i>c</i>	ND	ND	ND	ND
<i>Bst</i> NI	<i>i</i>	<i>i</i>	<i>i</i>	<i>i</i>	<i>h</i>	<i>h</i>	<i>h</i>	ND	ND	ND	ND
DNA G + C content (mol%)	56.4	56.4	56.4	55.1	55.7	55.1†	55.8‡	55.9†	60.3†	60.0†	59.8§

*For details, see Malimas *et al.* (2006) and Yukphan *et al.* (2004a, b).

†From Yukphan *et al.* (2004c).

‡From Tanasupawat *et al.* (2004).

§From Malimas *et al.* (2007).

nicotinic acid for growth. Restriction analysis of the 16S–23S rRNA gene ITS represents the *Gf* type, *d* type and *i* or *h* type of restriction patterns by digestion, respectively, with *Mbo*II, *Bsp*1286I, *Taq*I and *Bst*NI. Major ubiquinone is Q-10. DNA G + C contents are 55.1–56.4 mol%.

The type strain, NBRC 3271^T (=BCC 14458^T=strain 7^T, K. Kondo), was isolated from fruit of *Myrica rubra*, Chinese bayberry, and has a DNA G + C content of 56.4 mol% and is motile by means of polar flagella.

Acknowledgements

This study was supported in part by the Biodiversity Research and Training Program, Bangkok, Thailand.

References

- Asai, T. (1935). A taxonomic study and proposal of a new classification method of the acetic acid bacteria and allied oxidative bacteria that exist in fruit (continued). *J Agric Chem Soc Jpn* **11**, 674–708 (in Japanese).
- Asai, T., Iizuka, H. & Komagata, K. (1964). The flagellation and taxonomy of genera *Gluconobacter* and *Acetobacter* with reference to the existence of intermediate strains. *J Gen Appl Microbiol* **10**, 95–126.
- De Ley, J. (1961). Comparative carbohydrate metabolism and a proposal for a phylogenetic relationship of the acetic acid bacteria. *J Gen Microbiol* **24**, 31–50.

Ezaki, T., Yamamoto, N., Ninomiya, K., Suzuki, S. & Yabuuchi, E. (1983). Transfer of *Peptococcus indolicus*, *Peptococcus asaccharolyticus*, *Peptococcus prevotii*, and *Peptococcus magnus* to the genus *Peptostreptococcus* and proposal of *Peptostreptococcus tetradius* sp. nov. *Int J Syst Bacteriol* **33**, 683–698.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1983). Parsimony in systematics: biological and statistical issues. *Annu Rev Ecol Syst* **14**, 313–333.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (1995). PHYLIP (phylogeny inference package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Gosselé, F., Swings, J., Kersters, K. & De Ley, J. (1983). Numerical analysis of phenotypic features and protein gel electropherograms of *Gluconobacter* Asai 1935 emend. mut. char. Asai, Iizuka and Komagata 1964. *Int J Syst Bacteriol* **33**, 65–81.

Huong, V. T. L., Malimas, T., Yukphan, P., Potacharoen, W., Tanasupawat, S., Loan, L. T. T., Tanticharoen, M. & Yamada, Y. (2007). Identification of Thai isolates assigned to the genus *Gluconobacter* based on 16S–23S rDNA ITS restriction analysis. *J Gen Appl Microbiol* **53**, 133–142.

- Katsura, K., Yamada, Y., Uchimura, T. & Komagata, K. (2002). *Gluconobacter asaii* Mason and Claus 1989 is a junior subjective synonym of *Gluconobacter cerinus* Yamada and Akita 1984. *Int J Syst Evol Microbiol* **52**, 1635–1640.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Malimas, T., Yukphan, P., Takahashi, M., Potacharoen, W., Tanasupawat, S., Nakagawa, Y., Tanticharoen, M. & Yamada, Y. (2006). Heterogeneity of strains assigned to *Gluconobacter frateurii* Mason and Claus 1989 based on restriction analysis of 16S–23S rDNA internal transcribed spacer regions. *Biosci Biotechnol Biochem* **70**, 684–690.
- Malimas, T., Yukphan, P., Takahashi, M., Kaneyasu, M., Potacharoen, W., Tanasupawat, S., Nakagawa, Y., Tanticharoen, M. & Yamada, Y. (2007). *Gluconobacter kondonii* sp. nov., an acetic acid bacterium in the α -Proteobacteria. *J Gen Appl Microbiol* **53**, 301–307.
- Malimas, T., Yukphan, P., Takahashi, M., Kaneyasu, M., Potacharoen, W., Tanasupawat, S., Nakagawa, Y., Tanticharoen, M. & Yamada, Y. (2008). *Gluconobacter kondonii* Malimas et al. 2008. In *List of New Names and New Combinations Previously Effectively, but not Validly, Published*, Validation List no. 120. *Int J Syst Evol Microbiol* **58**, 529–530.
- Marmur, J. (1961). A procedure for isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Mason, L. M. & Claus, G. W. (1989). Phenotypic characteristics correlated with deoxyribonucleic acid sequence similarities for three species of *Gluconobacter*: *G. oxydans* (Henneberg 1897) De Ley 1961, *G. frateurii* sp. nov. and *G. asaii* sp. nov. *Int J Syst Bacteriol* **39**, 174–184.
- Micales, B. K., Johnson, J. L. & Claus, G. W. (1985). Deoxyribonucleic acid homologies among organisms in the genus *Gluconobacter*. *Int J Syst Bacteriol* **35**, 79–85.
- Saito, H. & Miura, K. (1963). Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim Biophys Acta* **72**, 619–629.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (editors) (1980). Approved lists of bacterial names. *Int J Syst Bacteriol* **30**, 225–420.
- Takahashi, M., Yukphan, P., Yamada, Y., Suzuki, K., Sakane, T. & Nakagawa, Y. (2006). Intrageneric structure of the genus *Gluconobacter* analyzed by the 16S rRNA gene and 16S–23S rRNA gene internal transcribed spacer sequences. *J Gen Appl Microbiol* **52**, 187–193.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Tanaka, M., Murakami, S., Shinke, R. & Aoki, K. (1999). Reclassification of the strains with low G+C contents of DNA belonging to the genus *Gluconobacter* Asai 1935 (*Acetobacteraceae*). *Biosci Biotechnol Biochem* **63**, 989–992.
- Tanasupawat, S., Thawai, C., Yukphan, P., Moonmangmee, D., Itoh, T., Adachi, O. & Yamada, Y. (2004). *Gluconobacter thailandicus* sp. nov., an acetic acid bacterium in the α -Proteobacteria. *J Gen Appl Microbiol* **50**, 159–167.
- Tanasupawat, S., Thawai, C., Yukphan, P., Moonmangmee, D., Itoh, T., Adachi, O. & Yamada, Y. (2005). *Gluconobacter thailandicus* Tanasupawat et al. 2005. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSEM*, List no. 103. *Int J Syst Evol Microbiol* **55**, 983–985.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Trček, J. & Teuber, M. (2002). Genetic and restriction analysis of the 16S–23S rDNA internal transcribed spacer regions of the acetic acid bacteria. *FEMS Microbiol Lett* **208**, 69–75.
- Verlander, C. P. (1992). Detection of horseradish peroxidase by colorimetry. In *Nonisotopic DNA Probe Techniques*, pp. 185–201. Edited by L. J. Kricka. New York: Academic Press.
- Yamada, Y. & Akita, M. (1984a). An electrophoretic comparison of enzymes in strains of *Gluconobacter* species. *J Gen Appl Microbiol* **30**, 115–126.
- Yamada, Y. & Akita, M. (1984b). *Gluconobacter cerinus*. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSE*, List no. 16. *Int J Syst Bacteriol* **34**, 503–504.
- Yamada, Y., Aida, K. & Uemura, T. (1969). Enzymatic studies on the oxidation of sugar and sugar alcohol. V. Ubiquinone of acetic acid bacteria and its relation to classification of *Gluconobacter* and *Acetobacter*, especially of the so-called intermediate strains. *J Gen Appl Microbiol* **15**, 186–196.
- Yamada, Y., Okada, Y. & Kondo, K. (1976). Isolation and characterization of “polarly flagellated intermediate strains” in acetic acid bacteria. *J Gen Appl Microbiol* **22**, 237–245.
- Yamada, Y., Itakura, N., Yamashita, M. & Tahara, Y. (1984). Deoxyribonucleic acid homologies in strains of *Gluconobacter* species. *J Ferment Technol* **62**, 595–600.
- Yamada, Y., Hosono, R., Lisdiyanti, P., Widyastuti, Y., Saono, S., Uchimura, T. & Komagata, K. (1999). Identification of acetic acid bacteria isolated from Indonesian sources, especially of isolates classified in the genus *Gluconobacter*. *J Gen Appl Microbiol* **45**, 23–28.
- Yamada, Y., Katsura, K., Kawasaki, H., Widyastuti, Y., Saono, S., Seki, T., Uchimura, T. & Komagata, K. (2000). *Asaia bogorensis* gen. nov., sp. nov., an unusual acetic acid bacterium in the α -Proteobacteria. *Int J Syst Evol Microbiol* **50**, 823–829.
- Yukphan, P., Potacharoen, W., Nakagawa, Y., Tanticharoen, M. & Yamada, Y. (2004a). Identification of strains assigned to the genus *Gluconobacter* Asai 1935 based on the sequence and the restriction analyses of the 16S–23S rDNA internal transcribed spacer regions. *J Gen Appl Microbiol* **50**, 9–15.
- Yukphan, P., Malimas, T., Takahashi, M., Potacharoen, W., Busabun, T., Tanasupawat, S., Nakagawa, Y., Tanticharoen, M. & Yamada, Y. (2004b). Re-identification of *Gluconobacter* strains based on the restriction analysis of the 16S–23S rDNA internal transcribed spacer regions. *J Gen Appl Microbiol* **50**, 189–195.
- Yukphan, P., Takahashi, M., Potacharoen, W., Tanasupawat, S., Nakagawa, Y., Tanticharoen, M. & Yamada, Y. (2004c). *Gluconobacter albidus* (ex Kondo and Ameyama 1958) sp. nov., nom. rev., an acetic acid bacterium in the α -Proteobacteria. *J Gen Appl Microbiol* **50**, 235–242.
- Yukphan, P., Potacharoen, W., Tanasupawat, S., Tanticharoen, M. & Yamada, Y. (2004d). *Asaia krungthepensis* sp. nov., an acetic acid bacterium in the α -Proteobacteria. *Int J Syst Evol Microbiol* **54**, 313–316.
- Yukphan, P., Takahashi, M., Potacharoen, W., Tanasupawat, S., Nakagawa, Y., Tanticharoen, M. & Yamada, Y. (2005). *Gluconobacter albidus* (ex Kondo and Ameyama 1958) Yukphan et al. 2005. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSEM*, List no. 103. *Int J Syst Evol Microbiol* **55**, 983–985.